JCM
Journals.ASM.org

Molecular and Clinical Diagnosis of Group A Streptococcal Pharyngitis in Children

Susanna Felsenstein,[a] Diala Faddoul,[a] Richard Sposto,[b] Kristine Batoon,[c] Claudia M. Polanco,[c] Jennifer Dien Bard[d]

Division of Infectious Diseases, Department of Pediatrics, Children's Hospital Los Angeles and Keck School of Medicine of University of Southern California, Los Angeles, California, USA[a]; Department of Preventive Medicine, Keck School of Medicine of University of Southern California, Los Angeles, California, USA[b]; Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, Los Angeles, California, USA[c]; Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles and Keck School of Medicine of University of Southern California, Los Angeles, California, USA[d]

Group A *Streptococcus* (GAS) pharyngitis is a very common condition causing significant morbidity in children. Accurate diagnosis followed by appropriate antimicrobial therapy is recommended to prevent postinfectious sequelae. Diagnosis of GAS pharyngitis by a rapid antigen detection test (RADT) or culture in the absence of discriminating clinical findings remains challenging. Validation of new sensitive rapid diagnostic tests is therefore a priority. The performance of a loop-mediated isothermal amplification (LAMP) assay (*illumi*gene assay) for the diagnosis of GAS pharyngitis was compared with that of a RADT and standard culture in 361 pediatric throat swab samples. Discrepant results were resolved using an alternate molecular assay. Test results were correlated with clinical presentations in patients positive by either method. The closest estimate of the true prevalence of GAS pharyngitis was 19.7% (71/361 samples). The *illumi*gene assay alone detected 70/71 GAS-positive samples; RADT and culture detected 35/71 and 55/71 samples, respectively. RADT followed by culture confirmation of RADT-negative specimens detected 58/71 cases. The *illumi*gene assay increased identification among children eligible for testing by American College of Physicians (ACP)/American Academy of Family Physicians (AAFP) criteria from 31 to 39 positive cases, five of which were false positives. Analysis of clinical data in GAS-positive patients indicated that a significantly greater proportion of children with McIsaac scores of ≥4 tested positive by the *illumi*gene assay versus RADT and culture. Overall, the *illumi*gene assay was much more sensitive and was similarly specific for GAS detection, compared to culture alone, RADT alone, or the ACP/AAFP RADT/culture algorithm. Combining high sensitivity with rapidly available results, the *illumi*gene GAS assay is an appropriate alternative to culture for the laboratory diagnosis of GAS pharyngitis in patients for whom testing is clinically indicated.

Group A *Streptococcus* (GAS) is a commonly encountered pathogen that causes a broad spectrum of diseases. Clinical features of GAS pharyngitis are indistinguishable from pharyngitis caused by other pathogens. Palatal petechiae and scarlatiniform rash, although highly specific, are rare (1). Early diagnosis and treatment are recommended to prevent suppurative and nonsuppurative postinfectious sequelae, such as peritonsillar abscesses, lymphadenitis, acute rheumatic fever (ARF), and poststreptococcal glomerulonephritis (2, 3). Current guidelines by the Infectious Diseases Society of America (IDSA) (3), the American Academy of Pediatrics (AAP) (4), and the American Heart Association (AHA) (5) recommend confirmation of GAS pharyngitis in children with a rapid antigen detection test (RADT), with follow-up cultures in RADT-negative cases (3, 5). The current IDSA guidance specifies that a throat culture should be performed for children with negative RADT results and treatment is indicated when the results of either test are positive. Clinical scoring systems, namely, Centor and McIsaac scores, integrate signs and symptoms to diagnose GAS pharyngitis (6, 7). Additionally, the McIsaac scoring system considers children 3 to 14 years of age to be at higher risk (8, 9). While the latest AAP guidelines recommend additional clinical findings to assist clinicians in determining when testing for GAS pharyngitis is indicated for children, the use of clinical scores remains a recommendation by the American College of Physicians (ACP) (7).

The microbiological gold standard for the diagnosis of GAS pharyngitis is culturing of pharyngeal swab specimens to screen for beta-hemolytic colonies. Although the sensitivity of cultures has been reported to be 90% to 95%, multiple variables can affect the yield, including specimen integrity, culture methods, and prior antibiotic use (10–12). In addition, culture can take up to 48 h, delaying appropriate antimicrobial treatment (13). Rapid diagnosis of GAS pharyngitis is provided by RADT. Although this technique is highly specific, its sensitivity is as low as 31 to 50% (11), prompting the need for back-up cultures (3, 5). Molecular methods may offer alternatives to improve speed and accuracy in the diagnosis of GAS pharyngitis and have been shown to have superior sensitivity and specificity (14–16). Herein, we present the performance of the *illumi*gene group A *Streptococcus* assay, a molecular assay for the diagnosis of GAS pharyngitis, in comparison with a RADT and a standard culture method, and we correlate the findings with clinical presentations in a pediatric cohort.

(Some of the study data were presented in poster format at the 113th General Meeting of the American Society for Microbiology, Denver, CO, 18 to 21 May 2013.)

Received 27 May 2014 Returned for modification 30 June 2014
Accepted 14 August 2014
Published ahead of print 20 August 2014
Editor: R. Patel
Address correspondence to Susanna Felsenstein, sfelsenstein@chla.usc.edu.
S.F. and D.F. are co-first authors.

doi:10.1128/JCM.01489-14

TABLE 1 Performance of RADT and *illumi*gene group A *Streptococcus* assay versus routine culture

Assay	No. of cases[a]				Performance result (95% CI) (%)			
	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV
RADT	32	3[b]	300	26[c]	55.2 (42.5–67.3)	99.1 (96.9–99.8)	91.4 (76.9–97.8)	92.0 (87.2–95.2)
*illumi*gene	54	26[d]	277	4[e]	93.1 (83.1–97.8)	91.4 (87.7–94.1)	67.5 (56.6–76.8)	98.5 (95.1–99.9)

[a] TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPV, positive predictive value; NPV, negative predictive value.

[b] All 3 cases were resolved by the *illumi*gene assay and PCR as true positive.

[c] Three of 26 cases were resolved by culture as true negative, and 23/26 cases were confirmed by the *illumi*gene assay as false negative.

[d] Ten of 26 cases were confirmed by PCR as false positive, 13/26 cases were resolved by PCR as true positive, and 3/26 cases were resolved by RADT as true positive.

[e] One of 4 cases was confirmed by PCR as false negative, and 3/4 cases were resolved by culture review as true negative.

MATERIALS AND METHODS

Study design. Two throat swab specimens each were collected from 361 pediatric patients who presented to the emergency department (ED) at Children's Hospital Los Angeles (CHLA) in December 2012 through March 2013. Throat specimens, which were collected using the CultureSwab collection and transport system (Becton Dickinson, Sparks, MD), were obtained at the physician's discretion by a registered pediatric nurse. According to institutional policy, throat swabs are ordered for children presenting with McIsaac scores of ≥2. For the purposes of this study, all throat swabs collected in the ED during the study period were included; therefore, patients who did not fulfill the criterion of a McIsaac score of ≥2 and who presented with fever of unknown origin, upper respiratory tract symptoms, or subjective complaints of throat pain or discomfort on swallowing were also included. This strategy permitted comparison of test performance over several grades of disease severity and with presumed carrier status. Clinical scores and presentations of the patients included are detailed in Results. One swab was used for the OSOM Ultra Strep A RADT (Sekisui Diagnostics, Lexington, MA) and the second swab for routine culture and the *illumi*gene GAS assay. All diagnostic tests were performed for each sample, and test performance results were compared between tests. Discrepant tests were additionally tested by PCR, as described below. The study was approved by the CHLA institutional review board.

Conventional diagnostic workup. For RADT, the OSOM Ultra Strep A assay was performed according to the manufacturer's protocol. A culture was set up with the second swab by inoculating a 5% sheep blood agar plate (BAP) and incubating the plate at 35°C to 37°C in ambient air for 24 to 48 h. BAPs were examined at 24 and 48 h for beta-hemolytic colonies. GAS was confirmed by the presence of Gram-positive cocci in chains with Gram staining, nonreactive catalase findings, and the presence of GAS antigen in the latex agglutination test (PathoDx strep grouping kit; Thermo Fisher Scientific, Waltham, MA). Culture plates growing GAS colonies were quantified as follows: few, growth in the first quadrant; moderate, growth in the second quadrant; many, growth in the third or fourth quadrant.

***illumi*gene group A *Streptococcus* assay.** After inoculation of the BAP, the swab was used to perform the *illumi*gene GAS assay, according to the manufacturer's protocol. Briefly, swab tips were broken into the sample preparation tubes, the tube contents were vortex-mixed for 10 s, and 10 drops of specimen were transferred to a heat treatment tube. The tube was incubated at 95°C for 10 min, and 50 μl of lysate was transferred to the test and control chambers. The test device was inserted into the *illumi*pro-10 incubator/reader for loop-mediated isothermal amplification (LAMP), targeting the highly conserved 206-bp sequence of the *Streptococcus pyogenes* pyrogenic exotoxin (*speB*) gene. Within 40 min, amplified product was detected as the presence of turbidity, due to precipitated magnesium pyrophosphate.

Performance and discrepancy analysis. The remnant heat treatment tubes were stored at −80°C, to allow laboratory-developed real-time (RT)-PCR assay of samples with discrepant results. The method employed TaqMan primers and probes directed against a different region of the *speB* gene than the *illumi*gene GAS assay (forward primer, 5′-TGTCAGTGTCAACTAACCGTGT-3′; reverse primer, 5′-CGGCAAATACTGGGTTAGCAAG-3′; probe, 5′-FAM-AGTAAGGAGGTGTGTCCAATGTACCGT-36-TAMSp-3′). RT-PCR was performed on a Rotor-Gene RT-PCR cycler (Qiagen, Germantown, MD) at 95°C for 10 min, followed by 60 cycles of 95°C for 15 s and 57°C for 60 s, with final extension at 72°C for 5 min. Cultures were also reassessed in cases with discrepancies, and identification of GAS was confirmed by repeat latex agglutination testing and the pyrrolidonyl arylamidase (PYR) test (Hardy Diagnostics, Santa Maria, CA).

Medical chart reviews. Clinical and laboratory data were collected retrospectively, by an investigator blinded to test outcomes, for all patients who tested positive for GAS by at least one diagnostic test. Variables collected included age, date of diagnosis, and clinical criteria, i.e., (i) fever (defined as a documented temperature of ≥38°C), (ii) absence of cough and rhinorrhea, (iii) presence of tonsillar exudate, and (iv) presence of swollen and/or tender anterior cervical lymph nodes. Centor and McIsaac scores were calculated (9, 17). Patients less than 3 years of age were excluded from analyses of the clinical scores, as neither scoring system has been validated for this age group.

Statistical analysis. Comparisons between two groups were performed using the *t* test if data were normally distributed and the Mann-Whitney test if they were not. The sample size of 361 was dictated by the number of patients satisfying the eligibility criteria during the study period; however, the study was sufficiently large to yield precise estimates of the overall concordance rate, with a standard error of 0.026 at most. Quantitative variables were expressed as mean and standard deviation (SD) if data were normally distributed and as median and interquartile range if they were not. Tests of association between categorical variables were based on the chi-square and Fisher's exact tests. For differences between paired proportions for the individual diagnostic tests, the McNemar test was used. Sensitivity, specificity, and predictive values were calculated using culture results or the best estimate of true GAS status as diagnostic gold standards, as indicated. Statistical computations were performed using SPSS 12.0 (SPSS Inc., Chicago, IL).

RESULTS

Evaluation of group A *Streptococcus* detection methods. When the RADT was compared with culture, 300/361 patients (83.1%) tested negative and 32/361 (8.9%) positive by both methods. An additional 26/361 patients (7.2%) tested positive by culture and 3 patients by RADT alone. This resulted in a sensitivity of 55.2% (95% confidence interval [CI], 42.5% to 67.3%) and a specificity of 99.1% (95% CI, 96.9% to 99.8%) for RADT (Table 1).

Alternatively, when routine culture was compared with the *illumi*gene GAS assay, both methods detected 54 positive cases (15.0%). The *illumi*gene GAS assay detected an additional 26 positive cases (7.2%) but missed 4 GAS cases detected by culture, 3 of which were also negative by RADT (Table 1). The sensitivity and specificity were 93.1% (95% CI, 83.1% to 97.8%) and 91.4% (95% CI, 87.7% to 94.1%), respectively, and the overall prevalence rate based on the *illumi*gene assay results was 22.2% (80/361 samples).

TABLE 2 Agreement of RADT, culture, and *illumi*gene group A *Streptococcus* assay results

Original test and second assay result	No. with second test result of: Negative	Positive	Agreement (95% CI) (%) Positive	Negative	Overall	P^a
RADT						
Culture negative	300	3	55.2 (42.5–67.3)	99.1 (96.9–99.8)	91.9 (88.7–94.4)	<0.001
Culture positive	26	32				
*illumi*gene assay						
Culture negative	277	26	93.1 (83.1–97.8)	91.4 (87.7–94.1)	91.7 (88.4–94.2)	<0.001
Culture positive	4	54				
RADT negative	280	46	97.1 (84.2–99.9)	85.9 (81.7–89.3)	86.9 (83.1–90.1)	<0.001
RADT positive	1	34				

[a] McNemar test.

The concordance of test results for the 361 samples is summarized in Table 2.

GAS colonies were quantified in 55/58 culture-positive specimens (94.8%). Positive RADT results were significantly associated with higher colony counts ($P < 0.001$). Fourteen culture-positive cases (25%) with moderate or many colonies had negative RADT results ($P < 0.001$). Positive *illumi*gene assay results were not associated with higher colony counts ($P = 0.47$), and the one false-negative case had a moderate colony count.

Discrepancy analysis. Among 30 cases with discrepancies between *illumi*gene GAS assay and culture results, the *illumi*gene GAS assay detected 26 additional GAS-positive specimens, compared with culture (Table 3). Three of 26 specimens were also RADT positive and were considered true-positive specimens. The BAPs were reviewed for the 4 *illumi*gene GAS assay-negative, culture-positive specimens; 3 BAPs revealed beta-hemolytic colonies that were mistakenly identified as GAS by routine methods, and the fourth BAP was confirmed to grow GAS colonies. Therefore, PCR assays were performed for 24 specimens with discrepant results, including 23 *illumi*gene GAS assay-positive, culture-negative specimens and one *illumi*gene GAS assay-negative, culture-positive specimen. Fourteen (58%) of 24 specimens were confirmed to be GAS positive and 10 were confirmed to be GAS negative by PCR.

Following discrepancy analysis, the adjusted sensitivity and specificity for the *illumi*gene GAS assay were 98.6% (95% CI, 91.7% to 99.9%) and 96.5% (95% CI, 93.6% to 98.2%), respectively. The positive predictive value was 87.5% (95% CI, 78.3% to 93.3%), and the negative predictive value was 99.6% (95% CI, 97.8% to 99.9%). After resolution of discrepancies, the final GAS pharyngitis prevalence rates in the entire cohort using RADT, culture, and *illumi*gene GAS assay were 9.7% (35/361 patients), 15.2% (55/361 patients), and 19.7% (71/361 patients), respectively. Thus, the best estimate of true prevalence in our patient population was 19.7% (Tables 2 and 3).

Correlation with clinical presentations. (i) Age distribution. Ages were normally distributed, with a mean age of 7.4 years (SD, 4.2 years) and an age range of 2 months to 18 years. Sixty-six of 361 children were ≤3 years of age. When cases positive by culture were compared with those positive by RADT ($P = 0.92$) or *illumi*gene GAS assay ($P = 0.60$), the mean ages did not differ between the groups.

(ii) Characterization of clinical scores for patients with positive GAS results. Clinical data were available for 75/81 patients (94%) who tested positive by at least one diagnostic test. Six of 75 children were ≤3 years of age and were analyzed separately; 2/6 tested positive by all three methods and 3/6 by culture and *illumi*gene GAS assay only. One patient tested positive by *illumi*gene GAS assay alone, which was confirmed by PCR. Therefore, all children ≤3 years of age had true-positive test results for GAS, resulting in a rate of 9% (6/66 patients) in this age group. Four of 6 patients presented with fever, lymphadenopathy, and exudates; all six patients had an absence of cough. The average age of the remaining 69 patients included in clinical score analysis was 8.4 years (SD, 3.5 years), with ages ranging from 4 to 18 years. Five of 69 patients were 15 years of age or older.

TABLE 3 Discrepancy analysis of *illumi*gene GAS assay results

Culture result	RADT result	*illumi*gene GAS assay result	PCR result	Status using ACP criteria	Reference GAS status	No. of cases (%)
Positive	Positive	Positive		Positive	True positive	31 (8.6)
Positive	Positive	Negative	Positive	Positive	True positive	1 (0.3)
Positive	Negative	Positive		Positive	True positive	23 (6.4)
Positive	Negative	Negative		Positive	True negative[a]	3 (0.8)
Negative	Positive	Positive		Positive	True positive[b]	3 (0.8)
Negative	Positive	Negative		Positive	True negative	0 (0.0)
Negative	Negative	Positive	Positive	Negative	True positive	13 (3.6)
Negative	Negative	Positive	Negative	Negative	True negative	10 (2.8)
Negative	Negative	Negative		Negative	True negative	277 (76.7)

[a] Review of BAPs revealed colonies of beta-hemolytic streptococci other than GAS.
[b] Based on positive RADT results.

TABLE 4 GAS-positive cases according to diagnostic method and McIsaac score[a]

Test result	No. (%) with McIsaac score of:				Total no.	P^b
	1 ($n = 8$)	2 ($n = 13$)	3 ($n = 27$)	≥4 ($n = 21$)		
Culture positive	4 (50)	10 (77)	18 (67)	14 (75)	46	0.68
RADT positive	3 (38)	4 (31)	14 (52)	9 (43)	30	0.64
*illumi*gene assay, all positive	8 (100)	13 (100)	26 (96)	21 (100)	68	0.80
Confirmed true positive	6 (75)	11 (85)	23 (89)	19 (91)	59	0.63
Confirmed false positive	2 (25)	2 (15)	3 (11)	2 (10)	9	0.62

[a] $n = 69$.
[b] Fisher's exact test for 2 × r tables.

Positive results with either test method were associated with higher clinical scores (Tables 4 and 5). Forty-nine (71%) of 69 patients were febrile, 27/69 patients (39%) had tonsillar exudates, 22/69 patients (32%) had cervical lymphadenopathy, and 48/69 patients (70%) had absence of cough or other viral symptoms. None of these clinical characteristics was associated with GAS-positive status by any method (data not shown).

Of the 20 patients included in the chart analysis who tested *illumi*gene GAS assay positive but culture and/or RADT negative, only one was <3 years of age. Seven of 20 patients had McIsaac scores of 4 or 5, 9/20 patients had scores of 2 or 3, and 4 patients had scores of 1. Confirmatory PCR assay results were negative for 2/7 patients scoring 4 or over, for 5/9 patients scoring 2 or 3, and for 2/4 patients scoring 1. Symptom severity by McIsaac scores did not differ significantly between patients positive by *illumi*gene GAS assay only and those positive by more than one modality ($P = 0.11$).

Treatment and diagnostic implications among patients for whom clinical information was retrieved. Applying the ACP/American Academy of Family Physicians (AAFP) strategy (18) to patients >3 years of age, empirical therapy without testing would have been indicated for 21/69 patients based on McIsaac scores. Of these 21 samples, all were positive by the *illumi*gene GAS assay, including two samples identified as false-positive specimens by PCR (Table 4). Only 9/21 samples were RADT and culture positive, and an additional 5 were culture positive only. Assuming that GAS was causative of the symptoms, the *illumi*gene GAS assay increased the yield in this subgroup from 14/21 to 19/21 true-positive cases, at the expense of identifying 2 false-positive cases. Culture-positive cases yielded colony quantitation of many in 10 cases and moderate in 4 cases.

Of the 8 children with McIsaac scores of 1, indicating a low likelihood of GAS pharyngitis (Table 4), none had fever, lymphadenopathy, or tonsillar exudate, and 6/8 had rhinorrhea and cough; 3/8 cases were RADT and culture positive, and one additional case was culture positive only. All 8/8 samples were *illumi*gene GAS assay positive, and 4/8 results were confirmed by PCR. The 3 cases that were positive with all three methods grew moderate or many colonies on BAPs, and the one RADT-negative, culture-positive case grew only a few colonies. Of the remaining patients, 40 had McIsaac scores of 2 or 3; 18/40 cases were RADT positive, and another 13 were identified by follow-up culture of RADT-negative specimens. The *illumi*gene GAS assay results were positive in 39/40 cases. Of these, PCR testing identified five as false-positive cases and one as a false-negative case. The false-negative specimen was RADT and culture positive, with a moderate colony count.

Application of Centor scores showed similar findings (Table 5). Of the 38 patients with scores of 2 or 3, 20 were RADT positive and an additional 8 were culture positive, resulting in 28 children who would have received empirical therapy. The *illumi*gene GAS assay identified 37/38 as positive. Among patients with Centor scores of 0 and 1, the *illumi*gene GAS assay identified 21/21; RADT identified 7/21 and culture 14/21 as positive, likely representing carrier status.

Additionally, we evaluated clinical data for culture- and RADT-negative specimens that were defined as true-negative or true-positive cases based on confirmatory PCR data alone. Among the 10 cases defined as true negative on the basis of PCR and *illumi*gene GAS assay results, two children had McIsaac scores of 4 or 5, five had McIsaac scores of 2 or 3, and three had McIsaac scores of 1. Conversely, among the 13 true-positive cases, six patients had scores of 4 or 5, four had scores of 2 or 3, and two had scores of 1. For the remaining patient, clinical data could not be retrieved. Thus, the cases defined as true positive were more likely to have high McIsaac scores than the true-negative cases.

DISCUSSION

The diagnosis of GAS pharyngitis continues to be a challenge, and clinical findings are notoriously nondiscriminative and unreliable. GAS is the most prevalent cause of bacterial pharyngitis, accounting for 5 to 15% of pharyngitis cases in adults and 20 to

TABLE 5 GAS-positive cases according to diagnostic method and Centor score[a]

Test result	No. (%) with Centor score of:				Total no.	P^b
	0 ($n = 6$)	1 ($n = 15$)	2 ($n = 25$)	≥3 ($n = 23$)		
Culture positive	2 (33)	12 (80)	16 (64)	16 (70)	46	0.24
RADT positive	2 (33)	5 (33)	12 (48)	11 (48)	30	0.75
*illumi*gene assay, all positive	6 (100)	15 (100)	25 (100)	22 (96)	68	0.47
Confirmed true positive	5 (83)	13 (87)	22 (88)	20 (87)	60	0.98
Confirmed false positive	1 (17)	2 (13)	3 (12)	2 (9)	8	0.80

[a] $n = 69$.
[b] Fisher's exact test for 2 × r tables.

30% in children (1, 19–22). In the United States, approximately 7.3 million outpatient visits are attributed to children with acute pharyngitis, and the overall societal cost of GAS pharyngitis ranges from $224 million to $539 million per year (23). Therefore, more accurate identification of GAS pharyngitis is of interest, particularly for children, among whom both carrier rates and incidence rates of GAS pharyngitis are higher (1, 3, 24, 25) and pathogens associated with clinically indistinguishable symptom complexes are common (26, 27).

Current diagnostic methods are unable to distinguish whether the presence of GAS reflects carriage or a cause of pharyngitis (28, 29). However, up to 20 to 37% of pediatric pharyngitis cases have been found to be culture positive for GAS (1, 3, 25). In our cohort, physician-led decisions to collect throat swabs were made, and the patients would be presumed to have been symptomatic. However, review of the clinical notes revealed that a significant proportion of patients had low clinical scores, presenting with symptoms consistent with viral illness. Testing might not have been warranted, as the risk of GAS pharyngitis in these cases is about 4% (4). Our study is the first to evaluate the feasibility of using a molecular assay for the diagnosis of GAS pharyngitis by correlating results with clinical presentations.

Detection and identification of GAS from pharyngeal swabs represent the current gold standard for the microbiological diagnosis of GAS pharyngitis, with reported sensitivities between 90 and 95% (10, 11). Optimal sampling of the posterior pharynx is imperative but is difficult in young children, and culture sensitivity can be as low as 20% with suboptimal sampling techniques (10). Discrepancies seen between culture and *illumi*gene GAS assay results in this study are unlikely to be due to sampling variability, as the same pediatric nurse collected the two swabs from each patient simultaneously. The 3 samples that were initially reported as beta-hemolytic colonies on BAPs were confirmed to be latex agglutination negative and PYR negative upon retesting. These 3 isolates were correctly identified as GAS negative by both the RADT and the *illumi*gene GAS assay. Three other samples were culture negative and both RADT and *illumi*gene GAS assay positive. In 2 cases, there was documentation of prior exposure to amoxicillin; in the third case, no information on antibiotic exposure prior to testing could be retrieved. These 3 cases were considered true-positive cases, as RADT has been reported to be highly specific and, according to current guidelines, confirmation is not required (3, 30). However, the fact that the results for these samples were not confirmed with the alternate PCR assay can be considered a limitation.

RADT has a significantly shorter turnaround time than culture and therefore is an attractive diagnostic option, especially in outpatient settings. While highly specific, RADT results showed concordance with positive culture results in only 55.2% of cases. Other studies similarly demonstrated low sensitivity of RADTs, compared to culture (31–33). The *illumi*gene GAS assay detected 13 additional true-positive cases, in comparison with RADT and/or culture, with sensitivity and specificity of 98.6% and 96.5%, respectively. Ten false-positive *illumi*gene GAS assay results were confirmed by PCR and may be attributed to poor specimen condition, since remnant samples after culture setup, *illumi*gene testing, and a freeze-thaw cycle were used for PCR confirmation. Therefore, the specificity of the *illumi*gene GAS assay may be even higher than reported here.

Recent studies investigating the performance characteristics of the *illumi*gene GAS assay for the diagnosis of GAS throat infections yielded similar results. A recent study comparing the performance of the *illumi*gene GAS assay with that of standard cultures, using remnant throat swab samples from a cohort not restricted to pediatric patients, yielded 100% sensitivity and 94% specificity, with a false-positive rate of 5% (14). However, clinical presentations and indications for testing were not analyzed. Similarly, another group reported a sensitivity of 100% and a specificity of 95.9% for the *illumi*gene GAS assay, in comparison with culture (34). Both studies incorporated laboratory-developed RT-PCR assays that were shown to be highly sensitive and specific (14, 34).

Depending on age and season, between 5 and 20% of asymptomatic children test positive for GAS (22, 35). Therefore, an argument against the higher number of positive results detected by molecular tests involves asymptomatic carriers. To determine whether the new positive cases exhibited signs and symptoms of GAS pharyngitis, clinical scores were determined for all positive patients. It was apparent that, while some guidelines exist for clinicians in the ED to determine whether screening for GAS pharyngitis would be valuable in guiding further management, these are not always adhered to, resulting in a number of patients being included in the study whose positive test results likely represent carrier status. Despite the fact that there was no correlation between RADT, culture, or molecular assay results and clinical scores, a finding consistent with other studies (6, 25), 16/20 patients with new positive results with the *illumi*gene GAS assay had McIsaac scores of $\geq$2, and 7 patients with scores of 4 presented with clinical signs and symptoms of GAS pharyngitis. Of note, only patients for whom the clinical decision was made to obtain throat swab specimens were included in this study; therefore, we cannot fully assess the impact of asymptomatic carrier status by molecular methods. We can confirm that all patients who were culture negative for GAS were also negative for group C and group G streptococci and *Arcanobacterium* spp. Conversely, the presence of alternative bacterial or viral agents causing the symptoms in patients with higher scores but negative microbiological diagnostic findings cannot be excluded.

Our study illustrates that, regardless of the diagnostic method utilized, appropriate clinical assessment must be employed to avoid detection of GAS carriers. Following the ACP/AAFP approach, 8/69 patients had McIsaac scores of only 1; of the remaining 61 patients eligible for testing, 45/61 patients (74%) would have received treatment based on positive RADT and/or culture results. If diagnostic testing with the *illumi*gene GAS assay alone had been used to guide treatment, 60/61 patients (99%) with McIsaac scores of $\geq$2 would have been identified as positive. Of these, 7/61 cases were false-positive by PCR confirmation. Therefore, while the *illumi*gene GAS assay is a highly sensitive assay, it is important to highlight that the use of any GAS diagnostic test requires adherence to clinical guidelines detailing the indications for testing. Nonadherence to these guidelines would contribute to the concerning increase in antibiotic use driven by positive test results, rather than a combination of clinical acumen and diagnostic tests.

The *illumi*gene GAS assay is a rapid molecular assay with high sensitivity and specificity and is demonstrated to be far superior to a RADT and culture for the detection of GAS in pharyngeal specimens. In addition, medical chart analysis of the *illumi*gene GAS assay-positive, RADT- and/or culture-negative patients revealed possible true-positive cases of GAS pharyngitis, rather than asymptomatic colonization, in a number of patients. Therefore, the *illumi*gene GAS assay proves to be a useful diagnostic tool for GAS pharyngitis when testing is appropriately limited to patients presenting with suggestive clinical symptoms in the absence of cough and coryza.

ACKNOWLEDGMENTS

We thank Meridian Biosciences, Inc., for supplying the *illumi*gene group A *Streptococcus* assay kits and the *illumi*pro-10 incubator reader for the study.

REFERENCES

1. **Ebell MH, Smith MA, Barry HC, Ives K, Carey M.** 2000. The rational clinical examination: does this patient have strep throat? JAMA **284:**2912–2918. http://dx.doi.org/10.1001/jama.284.22.2912.
2. **Wannamaker LW, Rammelkamp CH, Jr, Denny FW, Brink WR, Houser HB, Hahn EO, Dingle JH.** 1951. Prophylaxis of acute rheumatic fever by treatment of the preceding streptococcal infection with various amounts of depot penicillin. Am. J. Med. **10:**673–695. http://dx.doi.org/10.1016/0002-9343(51)90336-1.
3. **Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, Martin JM, Van Beneden C.** 2012. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin. Infect. Dis. **55:**e86–e102. http://dx.doi.org/10.1093/cid/cis629.
4. **Pickering LK, Baker CJ, Long SS, Kimberlin DW.** 2012. Red book: 2012 report of the Committee on Infectious Diseases, 29th ed. American Academy of Pediatrics, Elk Grove Village, IL.
5. **Gerber MA, Baltimore RS, Eaton CB, Gewitz M, Rowley AH, Shulman ST, Taubert KA.** 2009. Prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis: a scientific statement from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. Circulation **119:**1541–1551. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.191959.
6. **Le Marechal F, Martinot A, Duhamel A, Pruvost I, Dubos F.** 2013. Streptococcal pharyngitis in children: a meta-analysis of clinical decision rules and their clinical variables. BMJ Open **3:**pii=e001482. http://dx.doi.org/10.1136/bmjopen-2012-001482.
7. **Regoli M, Chiappini E, Bonsignori F, Galli L, de Martino M.** 2011. Update on the management of acute pharyngitis in children. Ital. J. Pediatr. **37:**10. http://dx.doi.org/10.1186/1824-7288-37-10.
8. **McIsaac WJ, White D, Tannenbaum D, Low DE.** 1998. A clinical score to reduce unnecessary antibiotic use in patients with sore throat. CMAJ **158:**75–83.
9. **Centor RM, Witherspoon JM, Dalton HP, Brody CE, Link K.** 1981. The diagnosis of strep throat in adults in the emergency room. Med. Decis. Making **1:**239–246.
10. **Fox JW, Marcon MJ, Bonsu BK.** 2006. Diagnosis of streptococcal pharyngitis by detection of *Streptococcus pyogenes* in posterior pharyngeal versus oral cavity specimens. J. Clin. Microbiol. **44:**2593–2594. http://dx.doi.org/10.1128/JCM.00797-06.
11. **Wegner DL, Witte DL, Schrantz RD.** 1992. Insensitivity of rapid antigen detection methods and single blood agar plate culture for diagnosing streptococcal pharyngitis. JAMA **267:**695–697. http://dx.doi.org/10.1001/jama.1992.03480050099033.
12. **Gerber MA, Spadaccini LJ, Wright LL, Deutsch L.** 1984. Latex agglutination tests for rapid identification of group A streptococci directly from throat swabs. J. Pediatr. **105:**702–705. http://dx.doi.org/10.1016/S0022-3476(84)80286-3.
13. **Snow V, Mottur-Pilson C, Cooper RJ, Hoffman JR.** 2001. Principles of appropriate antibiotic use for acute pharyngitis in adults. Ann. Intern. Med. **134:**506–508. http://dx.doi.org/10.7326/0003-4819-134-6-200103200-00018.
14. **Anderson NW, Buchan BW, Mayne D, Mortensen JE, Mackey TL, Ledeboer NA.** 2013. Multicenter clinical evaluation of the *illumi*gene group A *Streptococcus* DNA amplification assay for detection of group A *Streptococcus* from pharyngeal swabs. J. Clin. Microbiol. **51:**1474–1477. http://dx.doi.org/10.1128/JCM.00176-13.
15. **Pokorski SJ, Vetter EA, Wollan PC, Cockerill FR, III.** 1994. Comparison of Gen-Probe group A *Streptococcus* direct test with culture for diagnosing streptococcal pharyngitis. J. Clin. Microbiol. **32:**1440–1443.
16. **Uhl JR, Adamson SC, Vetter EA, Schleck CD, Harmsen WS, Iverson LK, Santrach PJ, Henry NK, Cockerill FR.** 2003. Comparison of LightCycler PCR, rapid antigen immunoassay, and culture for detection of group A streptococci from throat swabs. J. Clin. Microbiol. **41:**242–249. http://dx.doi.org/10.1128/JCM.41.1.242-249.2003.
17. **McIsaac WJ, Kellner JD, Aufricht P, Vanjaka A, Low DE.** 2004. Empirical validation of guidelines for the management of pharyngitis in children and adults. JAMA **291:**1587–1595. http://dx.doi.org/10.1001/jama.291.13.1587.
18. **Choby BA.** 2009. Diagnosis and treatment of streptococcal pharyngitis. Am. Fam. Physician **79:**383–390.
19. **Bisno AL.** 1996. Acute pharyngitis: etiology and diagnosis. Pediatrics **97:**949–954.
20. **Carapetis JR, Steer AC, Mulholland EK, Weber M.** 2005. The global burden of group A streptococcal diseases. Lancet Infect. Dis. **5:**685–694. http://dx.doi.org/10.1016/S1473-3099(05)70267-X.
21. **Kaplan EL, Top FH, Jr, Dudding BA, Wannamaker LW.** 1971. Diagnosis of streptococcal pharyngitis: differentiation of active infection from the carrier state in the symptomatic child. J. Infect. Dis. **123:**490–501. http://dx.doi.org/10.1093/infdis/123.5.490.
22. **Shulman ST.** 1994. Streptococcal pharyngitis: diagnostic considerations. Pediatr. Infect. Dis. J. **13:**567–571.
23. **Pfoh E, Wessels MR, Goldmann D, Lee GM.** 2008. Burden and economic cost of group A streptococcal pharyngitis. Pediatrics **121:**229–234. http://dx.doi.org/10.1542/peds.2007-0484.
24. **Shaikh N, Leonard E, Martin JM.** 2010. Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis. Pediatrics **126:**e557–e564. http://dx.doi.org/10.1542/peds.2009-2648.
25. **Shaikh N, Swaminathan N, Hooper EG.** 2012. Accuracy and precision of the signs and symptoms of streptococcal pharyngitis in children: a systematic review. J. Pediatr. **160:**487–493.e3. http://dx.doi.org/10.1016/j.jpeds.2011.09.011.
26. **Bisno AL.** 2001. Acute pharyngitis. N. Engl. J. Med. **344:**205–211. http://dx.doi.org/10.1056/NEJM200101183440308.
27. **Wessels MR.** 2011. Clinical practice: streptococcal pharyngitis. N. Engl. J. Med. **364:**648–655. http://dx.doi.org/10.1056/NEJMcp1009126.
28. **Dunne EM, Marshall JL, Baker CA, Manning J, Gonis G, Danchin MH, Smeesters PR, Satzke C, Steer AC.** 2013. Detection of group A streptococcal pharyngitis by quantitative PCR. BMC Infect. Dis. **13:**312. http://dx.doi.org/10.1186/1471-2334-13-312.
29. **Gerber MA, Ryan RW, Tilton RC, Watson JE.** 1984. Role of *Chlamydia trachomatis* in acute pharyngitis in young adults. J. Clin. Microbiol. **20:**993–994.
30. **Leung AK, Newman R, Kumar A, Davies HD.** 2006. Rapid antigen detection testing in diagnosing group A β-hemolytic streptococcal pharyngitis. Expert Rev. Mol. Diagn. **6:**761–766. http://dx.doi.org/10.1586/14737159.6.5.761.
31. **Armengol CE, Schlager TA, Hendley JO.** 2004. Sensitivity of a rapid antigen detection test for group A streptococci in a private pediatric office setting: answering the Red Book's request for validation. Pediatrics **113:**924–926. http://dx.doi.org/10.1542/peds.113.4.924.
32. **Chapin KC, Blake P, Wilson CD.** 2002. Performance characteristics and utilization of rapid antigen test, DNA probe, and culture for detection of group A streptococci in an acute care clinic. J. Clin. Microbiol. **40:**4207–4210. http://dx.doi.org/10.1128/JCM.40.11.4207-4210.2002.
33. **Gerber MA.** 1989. Comparison of throat cultures and rapid strep tests for diagnosis of streptococcal pharyngitis. Pediatr. Infect. Dis. J. **8:**820–824. http://dx.doi.org/10.1097/00006454-198911000-00032.
34. **Henson AM, Carter D, Todd K, Shulman ST, Zheng X.** 2013. Detection of *Streptococcus pyogenes* by use of *illumi*gene group A *Streptococcus* assay. J. Clin. Microbiol. **51:**4207–4209. http://dx.doi.org/10.1128/JCM.01892-13.
35. **Martin D.** 2004. XV Lancefield Symposium on Streptococci and Streptococcal Diseases. Indian J. Med. Res. **119**(Suppl):ix–xi.